Alcohol and Coronary Artery Calcification: An Investigation Utilizing Alcohol Flushing as an Instrumental Variable

Running title: Alcohol flushing response and coronary calcification

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Word count for main text plus abstract but not including tables: 4,100

No. of references: 47

Disclosures: None to declare.

Abbreviations:

ALDH2: acetaldehyde dehydrogenase 2

ALT: alanine aminotransferase

AST: aspartate aminotransferase

BMI: body mass index

CAC: coronary artery calcium

CI: confidence interval

GGT: gamma-glutamyl transferase

HDL-C: high-density lipoprotein-cholesterol

HOMA-IR: homeostasis model assessment of insulin resistance

IV: instrumental variable

LDL-C: low-density lipoprotein-cholesterol

OR: odds ratio

Abstract

Background: We examined whether alcohol flushing could be used as an instrumental variable (IV) and investigated the effect of alcohol consumption on coronary calcification using alcohol flushing status as an IV.

Methods: We analyzed cross-sectional data from 24,681 Korean adults (20,696 men and 3,985 women) who had been administered a questionnaire assessing alcohol consumption and alcohol flushing, as well as a coronary artery calcium (CAC) measurement. The associations of alcohol flushing status with potential confounders and alcohol consumption were examined. We employed the two-stage predictor substitution methodology for the IV analysis.

Results: The prevalence of alcohol flushing did not differ depending on gender, education, household income, cigarette smoking, or physical activity. Balanced levels of confounders were observed between alcohol flushers and non-flushers. Alcohol flushing was closely related to alcohol consumption and levels of liver enzymes. In men, a doubling in alcohol consumption was associated with increased odds of coronary calcification in both the IV analysis (odds ratio [OR] of CAC scores of 1 or over=1.11; 95% confidence interval [CI]=1.03–1.20) and the multivariable regression analysis (OR=1.04; 95% CI=1.01–1.07). For cardiovascular risk factors, the IV analysis showed a positive association between alcohol consumption and blood pressure and high-density lipoprotein-cholesterol.

Conclusions: Alcohol flushing can be used as an IV in studies evaluating the health impact of alcohol consumption, especially in East Asian countries. Through such an analysis, we found that increased alcohol consumption was associated with an increased risk of subclinical coronary atherosclerosis.

Keywords: Alcohol consumption; ALDH2; Cardiovascular disease, Coronary atherosclerosis; Mendelian randomization analysis; Republic of Korea

Key messages

- Alcohol flushing can be used as an instrumental variable (IV) in exploring the relationship between alcohol consumption and health outcomes.
- In the IV analysis using alcohol flushing, we found that increased alcohol consumption was associated with an increased risk of coronary calcification.
- The wider use of alcohol flushing questions in East Asian countries would produce better causal evidence regarding the health impacts of alcohol consumption.

Introduction

Alcohol use is considered one of the leading contributors to the global burden of disease.[1] The causal impact of alcohol consumption on coronary heart disease and conditions related to atherosclerosis has not yet established, despite its public health importance and the numerous studies that have addressed this issue.[2-5] Although randomized controlled trials on the effects of alcohol consumption on cardiovascular risk factors have been conducted,[4, 6, 7] it is difficult to implement such trials in a way that produces sustained large differences in alcohol consumption. Studies using genetic information as instrumental variables (IVs), such as Mendelian randomization studies,[8] have presented better evidence regarding the relationship between alcohol consumption and cardiovascular outcomes.[9-14] Among East Asians, including Chinese, Japanese, and Koreans, variants in the acetaldehyde dehydrogenase 2 (ALDH2) gene, which is responsible for the oxidation of acetaldehyde to acetate, are common,[15, 16] and can be used as an IV for Mendelian randomization studies (Supplementary Fig. 1).[11, 12, 14, 17] Several recent meta-analyses using case-control genetic studies have found that the mutant allele of ALDH2 that produces an inactive form of the ALDH2 enzyme are associated with coronary artery disease and myocardial infarction.[18-20] However, it is unclear whether these associations are mediated by alcohol use. Meanwhile, several Mendelian randomization studies showed that carriers of the mutant allele of this gene have lower levels of blood pressure,[11-14, 17] and high-density lipoprotein-cholesterol (HDL-C),[12-14, 17] and higher fasting blood glucose, and triglyceride levels.[14] However, only a limited number of studies using ALDH2 genetic variation as an IV have been carried out, possibly owing to the need for genetic information on large sample sizes.[21]

The alcohol flushing response, also called Asian flush or Asian glow, is associated with high acetaldehyde levels and predominantly results from an inherited deficiency in the ALDH2

enzyme among East Asians.[22] Questions about alcohol flushing have been used to identify ALDH2-deficient subjects and have been found to be highly reliable in detecting subjects with inactive ALDH2 in East Asian countries,[23, 24] including South Korea.[25] Alcohol flushing can be used as a proxy for ALDH2 genetic variation. If alcohol flushing is associated with lower levels of alcohol consumption and is independent of other confounders, analyses using alcohol flushing as an IV can produce better evidence regarding the relationship between alcohol consumption and health outcomes (Supplementary Fig. 1). In this study using South Korean subjects, we examined whether alcohol flushing can be used as an IV in exploring the causal impacts of alcohol consumption on health outcomes. We also investigated whether alcohol consumption affects coronary calcification, which is a sensitive metric for identifying subclinical atherosclerosis,[26] by using questions about alcohol flushing as an IV.

Methods

Study subjects

The study sample consisted of individuals who underwent cardiac computed tomography (CT) imaging for the assessment of coronary artery calcium (CAC) scoring as an optional test as part of a comprehensive health screening program at the Kangbuk Samsung Hospital, South Korea, between January 2012 and December 2013 (N=32,960). In Korea, the Industrial Safety and Health Law requires employees to participate in annual or biennial health examinations, offered free of charge. More than 80% of the study participants were employees of various companies and local governmental organizations or the spouses of these. CAC scoring has become a common CVD screening test in Korea.[27, 28] The remaining participants were people who volunteered for screening examinations.

Of a total of 32,960 individuals, we excluded participants who met any of the following

criteria (see Supplementary Fig. 2): 1) lifetime never-drinkers (n=1,149) who were not asked about alcohol flushing status; 2) participants with missing data in their responses about alcohol flushing (n=2,920); 3) those with any history of heart disease or stroke (n=340) considering the possibility of reverse causation; 4) participants with missing data about their level of alcohol consumption (n=475); 5) participants missing data on smoking, education, or physical activity (n=3,263); and 6) outlier data for alcohol consumption (≥ 120 g/day [n=132]), which we considered an implausible amount for daily consumption. Analyses using the outlier data for alcohol consumption produced similar findings as reported here (data not shown). The total number of eligible subjects was 24,681 (20,696 men and 3,985 women). The number of male subjects was greater than the number of female subjects since women of child-bearing age tended not to choose to undergo the CAC test due to concerns about radiation exposure. This study was approved by the Institutional Review Board of the Kangbuk Samsung Hospital, which exempted the requirement for informed consent because the study only involved the retrospective analysis of de-identified data.

Measurement of CAC by multidetector CT

CT scanning was performed with a LightSpeed VCT XTe 64-slice multidetector CT scanner (GE Healthcare, Tokyo, Japan) using the standard scanning protocol: 2.5-mm thickness, 400-ms rotation time, 120-kV tube voltage, and 124-mAs (310 mA·0.4 s) tube current under electrocardiogram-gated dose modulation. The quantitative CAC scores were calculated according to the method described by Agatston et al.[29] The interobserver and intraobserver reliabilities for the CAC scores were both excellent (intraclass correlation coefficient = 0.99).[30] CAC scores were categorized into 0, ≤100, and >100, which is a robust marker of coronary atherosclerotic burden and a predictor of future cardiovascular disease events.[31]

Measurement of alcohol flushing and alcohol consumption

An individual who had ever drunk any alcoholic beverage during his or her lifetime was defined as an ever-drinker. Ever-drinkers were asked about alcohol flushing using the following question: "Do you have a tendency to develop facial flushing immediately after drinking as little as one alcoholic drink?" The response categories were yes or no. Ever-drinkers who answered "yes" to the alcohol flushing question were classified as alcohol flushers and those who answered "no" were classified as alcohol non-flushers.

The questions about alcohol intake included the weekly frequency of alcohol consumption and the usual daily amount of consumption. Levels of alcohol consumption were categorized as none, <8 g/day, 8–30 g/day, and >30 g/day. Eight grams of alcohol are contained in one glass of soju, the most popular alcoholic beverage in South Korea. In our multivariable regression and IV analyses that examined the effect of alcohol consumption on various outcomes, we used a logarithmic continuous variable for alcohol intake per day: $\log_2$[(alcohol amount in grams + 1)/8]. A log-transformed variable was used because approximately 10% of participants reported no alcohol consumption and the distribution of alcohol consumption, among those who consumed alcohol, was markedly right-skewed. Since we used a log-transformed alcohol variable, the results of our analyses present changes in outcome variables per doubling of alcohol consumption.

Measurement of other variables

Data on demographic characteristics, education, household income, cigarette smoking status, physical activity, medical history, and medication use were also collected by standardized, self-administered questionnaires, as described elsewhere.[28, 32] Education levels

were categorized as less than elementary school, middle or high school graduation, and college graduation or higher. Monthly income levels were categorized as <4,000 USD, 4,000–5,999 USD, and ≥6,000 USD. Participants were categorized by smoking status as never, former, or current smokers. Physical activity levels were classified into three categories: inactive, minimally active, and health-enhancing physically active (HEPA), using the short form of the Korea-validated version of the International Physical Activity Questionnaire [33, 34] and standardized metabolic equivalents.

Height and weight were measured by trained nurses with the participants wearing a lightweight hospital gown and no shoes, and the body mass index (BMI) was calculated in units of kg/m^2. Blood pressure was measured using an automated vital signs monitor (53000, Welch Allyn, New York, USA) while subjects were in a sitting position with the arm supported at heart level. Blood samples were taken from the antecubital vein after at least a 10-hour fast. Serum total cholesterol and triglyceride levels were determined using an enzymatic colorimetric assay; low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were determined using a homogeneous enzymatic colorimetric assay. Fasting blood glucose levels were measured using the hexokinase method on the Cobas Integra 800 apparatus (Roche Diagnostics, Tokyo, Japan). Serum insulin was measured with an electrochemiluminescence immunoassay on a Modular Analytics E170 apparatus (Roche Diagnostics, Tokyo, Japan). Insulin resistance was assessed with the homeostasis model assessment of insulin resistance (HOMA-IR) according to the following equation; fasting blood insulin (μU/mL) × fasting serum glucose (mmol/L)/ 22.5. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by photometry using a Modular Analytics D2400 (Roche Diagnostics, Tokyo, Japan). Serum gamma-glutamyl transferase

(GGT) levels were determined using an enzymatic colorimetric assay on the Modular Analytics P800 apparatus (Roche Diagnostics, Tokyo, Japan). The Laboratory Medicine Department of the Kangbuk Samsung Hospital in Seoul, Korea has been accredited by the Korean Society of Laboratory Medicine and the Korean Association of Quality Assurance for Clinical Laboratories. The laboratory has also participated in the College of American Pathologists' Survey/Proficiency Testing program.

Statistical analyses

All analyses were conducted with SAS version 9.3 (SAS Institute, Cary, NC, USA). Levels of alcohol consumption, triglycerides, glucose, insulin, and HOMA-IR were log-transformed due to the skewed distributions of the measures. We analyzed the association of alcohol flushing status with confounders to examine if alcohol flushing status is independent of covariates other than alcohol consumption and alcohol consumption-related liver enzymes. We employed the standardized difference to check the IV assumption in examining associations of alcohol flushing with various potential confounders. The standardized difference is an intuitive index measuring effect size between two groups and a unified approach to quantifying the magnitude of difference between two groups.[35] Compared to a t-test or Wilcoxon rank-sum test, the standardized difference is independent of sample size and thus a useful statistical method for assessing balance, especially when statistical tests are conducted with large sample sizes. The standardized difference is calculated as a percentage with the same implication for both continuous and categorical variables. For continuous variable, the standardized difference was calculated as the absolute difference in mean divided by the square root of the averaged standard deviation, while, for categorical variable, a multivariate Mahalanobis distance method[35] to generalize the standardized difference metric was used. It has been suggested that a

standardized difference of <10% likely denotes a negligible imbalance.[36-38] We then analyzed the association of alcohol consumption and associated phenotypes, such as AST, ALT, and GGT, with alcohol flushing status to determine whether alcohol flushing status was a good predictor of alcohol consumption. For log-transformed variables, mean differences on the log scale were converted to percentage differences. We used IV analysis and multivariable linear regression analysis to evaluate the relationship of alcohol consumption with cardiovascular risk factors and CAC scores. We employed a log-transformed continuous variable for alcohol consumption in these analyses. In the multivariable regression analysis, we adjusted for the following confounders: age, socioeconomic status, smoking, physical activity, adult height, and medications for diabetes, hypertension, and dyslipidemia. For continuous and categorical outcomes, we used linear regressions and generalized and ordinal logistic regressions, respectively. In the IV analyses, we used the two-stage predictor substitution methodology,[39] which is the extension of the two-stage least squares method to nonlinear models. First, we performed linear regression in order to assess how log-transformed alcohol consumption was predicted by the instrumental variable. Subsequently, second-stage regressions were conducted for cardiovascular risk factors and CAC scores after replacing the log-transformed alcohol consumption variables with their predicted values. The same confounders were adjusted for in the IV analysis as in the multivariable regression analyses, considering any possibility of residual confounding. We also presented the IV analysis results without additional adjustment for confounders. We also conducted several additional analyses in order to obtain a more comprehensive understanding of the data. We analyzed the association of alcohol flushing with various age groups, and the demographic characteristics of study subjects were compared by levels of alcohol consumption. We also presented the associations of alcohol flushing status with cardiovascular risk factors and CAC scores. Lifetime never-drinkers were also compared

with ever-drinkers (see Supplementary Materials).

Results

Table 1 presents the characteristics of study subjects according to alcohol flushing status in men and women. Alcohol flushers accounted for 28.7% and 29.0% of men and women, respectively. In men, no meaningful differences in age, education, monthly household income, or cigarette smoking were detected between alcohol flushers and non-flushers. Although the P-values of the associations of alcohol flushing with physical activity and medication usage were <0.05 in men, no variables showed more than a 10% standardized difference of the mean. All variables among women showed less than a 10% standardized difference of the mean. Supplementary Table 1 shows similar prevalence rates of alcohol flushing by age group in men and women. Meanwhile, alcohol consumption was influenced by covariates including sociodemographic factors and health behaviors (Supplementary Table 2).

Table 2 shows the associations of alcohol flushing with alcohol consumption and liver enzymes related to alcohol use. In both men and women, alcohol flushers recorded lower levels of alcohol consumption than alcohol non-flushers. For example, 13.5% of male alcohol flushers consumed no alcohol in a week, while only 5.3% of male alcohol non-flushers did so. Among both men and women, approximately four times as many alcohol non-flushers consumed >30 g of alcohol per day than alcohol flushers. In men, 14.2% of alcohol flushers and 15.5% of alcohol non-flushers had a CAC score >0, whereas among women, the proportion was 2.3% among alcohol flushers and 3.3% among alcohol non-flushers.

Table 3 presents the results from the IV analysis and the multivariable regression analysis in men. Both analyses produced similar findings regarding the relationship of alcohol consumption with cardiovascular risk factors and CAC scores. However, the magnitude of the

relationship was generally greater in the IV analysis than in the multivariable regression analysis. In the IV analysis, cardiovascular risk factors other than LDL-C and HbA1C increased as the amount of alcohol consumption doubled. Similar results were found in the multivariable analysis, in which the relationships of alcohol consumption with log-transformed insulin and HOMA-IR values were smaller in magnitude than those obtained in the IV analysis. Both IV analysis and multivariable regression analysis showed that increased alcohol consumption was associated with an increased likelihood of coronary calcification. For example, adjusted IV analysis indicated that a doubling of alcohol consumption was associated with a 10.9% increase in the likelihood of a CAC score ≥1. The IV analysis using ordinal numbers for the outcome variables (0–3, corresponding to the categories of CAC scores) also showed strong associations of alcohol consumption with CAC. Supplementary Table 3 presents the associations of alcohol flushing status with cardiovascular risk factors and CAC scores in men and women.

Table 4 shows the IV and multivariable regression analyses for women. According to the IV analysis, diastolic blood pressure, HDL-C and log-transformed fasting glucose were positively associated with alcohol consumption. The multivariable analysis found positive associations of alcohol consumption with BMI, waist circumference, systolic and diastolic blood pressure, and HDL-C. However, the multivariable analysis showed a negative association between alcohol consumption and HbA1C levels. Table 4 presents the IV analysis for coronary calcification, in which an increased likelihood of coronary calcification was found with each doubling of alcohol consumption. Multivariable analysis showed that a doubling of alcohol consumption was associated with an increased likelihood of CAC scores ≥1. Considering the small number of CAC scores ≥101 (Supplementary Table 3), general and ordinal logistic regression analyses were not conducted in women.

Discussion

This study showed that alcohol flushing can be used as an IV for examining the relationship between alcohol consumption and health outcomes. The prevalence of alcohol flushing did not differ according to gender, education, monthly household income, cigarette smoking, or physical activity (Table 1), although these confounders were strongly associated with the amount of alcohol consumed (Supplemental Table 2). All confounders examined in this study displayed a standardized difference of the mean of less than 10%, indicating balanced levels of the confounders between alcohol flushers and non-flushers.[36-38] Supplemental Table 1 also presents findings indicating that the prevalence of alcohol flushing was relatively similar among all age groups, whereas age was associated with alcohol consumption. In addition, alcohol flushing was very closely related with alcohol consumption and associated phenotypes, including AST, ALT, and GGT especially in men.

Prior genetic studies have shown that alcohol flushing is a good proxy for ALDH2 deficiency. Two Japanese studies showed that alcohol flushing had a sensitivity and specificity of approximately 90% for predicting ALDH2 genetic variants.[23, 24] A prior Korean study reported sensitivity and specificity values of 94%.[25] Several studies that sampled different groups have consistently found the prevalence of ALDH2 heterozygotes to be approximately 27%–28% in South Korea.[15, 16, 25, 40] These studies have also found that ALDH2 null variants accounted for approximately 2%–6% of the South Korean population. In this study, the prevalence of alcohol flushing was 29%, which was fairly consistent in both genders and similar to the prevalence of ALDH2 heterozygotes among Koreans.

The findings of this study suggest that higher levels of alcohol consumption are associated with an increased risk of coronary calcification, especially among men. Analyses using alcohol flushing as an IV provided better evidence regarding the causal relationship of

alcohol consumption with cardiovascular outcomes, including coronary calcification. Although similar results were produced by the multivariable regression analysis, the magnitude of association was greater in the IV analysis. Doubled alcohol consumption was associated with a 10.9% increase in the likelihood of a CAC score ≥1 among men in the IV analysis adjusted for confounding variables, compared to a 3.9% increase in the multivariable analysis. In addition, generalized logistic regression analysis using ordinal outcome variables (0–3 for the categories of CAC scores) also showed that alcohol consumption affected coronary calcification in men.

Regarding cardiovascular risk factors, our IV analysis showed a positive association of alcohol consumption with blood pressure (both systolic and diastolic blood pressure for men and diastolic blood pressure for women), HDL-C in both men and women, and fasting blood glucose and triglyceride levels in men. Prior Asian studies using ALDH2 genetic variation as an IV have found similar results for these cardiovascular biomarkers,[10-14, 17] although Mendelian randomization studies of populations of European descent using alcohol dehydrogenase (ADH1B or ADH1C) genetic variation found no association between alcohol consumption and HDL-C levels,[9] and an inverse association with triglyceride.[9, 10] A prior meta-analysis of genome-wide association studies also showed an association between ALDH2 genetic variants and HDL-C in individuals of Asian ancestry.[41, 42] In addition, our IV analysis found positive associations of alcohol consumption with metrics of general (BMI) and abdominal obesity (waist circumference), especially in men. Prior Mendelian randomization studies have found similar associations for obesity metrics.[9, 10, 13, 14] We found a positive association between alcohol consumption and log-transformed fasting glucose in both men and women. This finding was unexpected because prior Mendelian randomization studies among both European and Asian populations have not found any such associations.[9, 10, 12, 13, 17] A recent Mendelian randomization study using South Korean samples showed that alcohol consumption adversely

affects fasting blood glucose.[14] Our IV analysis also revealed positive associations of alcohol consumption with log-transformed insulin and HOMA-IR in men but not in women, which were not reported in the prior Mendelian randomization studies. Gender differences in the amount and duration of alcohol consumption as well as macronutrients taken along with alcohol may explain the findings on effects of alcohol consumption on insulin secretion and insulin resistance.[43]

The magnitude of the associations was generally greater in the IV analysis than in the multivariable analysis. This was true among both men and women and also held true both for CAC scores and cardiovascular risk factors (Table 3, Table 4). Using our causal analysis might indicate the actual harm of alcohol use as manifested in coronary atherosclerosis. Although we adjusted for a wide range of confounders of the relationship between alcohol consumption and CAC in the multivariable analysis, unmeasured confounders or reverse causation might have reduced the magnitude of the association. This study considered only a limited subset of the lifetime exposure to socioeconomic disadvantages and underlying health problems that affect adulthood alcohol consumption behaviors.[44-46] Further adjustment for other covariates (BMI, blood pressure, LDL-C, and log-transformed glucose) resulted in attenuated associations of alcohol consumption with CAC in the multivariable analysis (data not shown).

A major contribution of this study is that we suggested the possibility of using alcohol flushing as an IV for examining the relationship between alcohol consumption and health outcomes. To the best of our knowledge, no prior study has used alcohol flushing status as an IV. Using CAC scores as an outcome variable is another strength of this study, as the CAC score is an established and reliable marker for coronary atherosclerosis[27] and is predictive of the incidence of cardiovascular events.[47] This study also has limitations. One important IV assumption of this study is that the alcohol flushing response (associated with exposures to

accumulated acetaldehyde) has no direct effect on the risk of coronary atherosclerosis. The acetaldehyde accumulation in blood after ethanol ingestion has short-term cardiovascular effects such as increases in heart rate and vasodilation (which leads to alcohol flushing).[48] The long-term effects of acetaldehyde on esophageal cancer are also established.[22, 49] However, the long-term direct effect of acetaldehyde on coronary atherosclerosis is not well understood. If this long-term direct effect is meaningful, alcohol flushing cannot be used as an IV. Since the sample size for women was small, sufficient cases with high CAC levels were not available. While several questionnaires have been used to assess alcohol flushing status,[22-25] the alcohol flushing questions used in this study were not directly validated with a genetic study. A comparative study using both ALDH2 and alcohol flushing as IVs would be required to enhance the understanding of the causal impact of alcohol on health in East Asians. In this study, information on alcohol flushing status was not obtained for lifetime never-drinkers, since they could not meaningfully respond to the alcohol flushing question. Lifetime drinking status showed patterns according to gender and other covariates (Supplementary Table 4). The unadjusted analyses showed worse findings for several cardiovascular risk factors and CAC scores among lifetime never-drinkers, with the specific findings differing by gender (Supplementary Table 5). These findings suggest that sickness makes people stop drinking, and that true lifetime never-drinkers choose that behavior based on underlying health problems existing at the time when people generally start drinking.[44-46] Moreover, relatively few cases of coronary calcification were observed in women, which hindered further analysis.

In conclusion, this study showed that alcohol flushing can be used as an IV for examining the health impacts of alcohol consumption. In the IV analysis, we found that increased alcohol consumption was associated with an increased risk of coronary calcification. Considering the paucity of genetic information in survey data related to alcohol metabolism

and consumption, compared to the ease of implementation of alcohol flushing questions in surveys, the wider use of alcohol flushing questions in East Asian countries would produce better causal evidence regarding the health impacts of alcohol consumption. Further studies using alcohol flushing as an IV are warranted in order to explore causal relationships between alcohol consumption and health outcomes.

Acknowledgments

We would like to thank Jiin Ahn (Kangbuk Samsung Hospital, Center for Cohort Studies, Seoul, Korea) for her technical support in data collection. YHK was supported by National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (No. NRF-2014R1A2A1A11051392).

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Table 1. Characteristics of study subjects (Korean adults) according to alcohol flushing status and gender.

Variables	Men					Women				
	Ever-drinkers (n=20 696)	Alcohol flushers (n=5940)	Alcohol non-flushers (n=14 756)	P-value	SD (%)	Ever-drinkers (n=3985)	Alcohol flushers (n=1155)	Alcohol non-flushers (n=2830)	P-value	SD (%)
Age (years)[a]	40.9 (7.0)	41.0 (7.2)	40.9 (6.9)	0.364	1.4	39.9 (7.5)	39.6 (7.5)	40.1 (7.5)	0.028	7.7
Education[b]										
Elementary or less	0.2	0.3	0.2	0.084	3.5	1.7	2.0	1.6	0.670	3.1
Middle or high school education	11.5	10.7	11.8			25.0	24.9	25.1		
College or over	88.3	89.1	88.0			73.3	73.2	73.3		
Monthly household income[b]										
<4,000 USD	20.5	20.4	20.2	0.675	1.9	21.1	22.4	20.5	0.077	9.2
4,000–5,999 USD	33.2	33.2	33.0			28.0	29.4	27.4		
≥6,000 USD	33.2	33.8	33.6			31.0	28.1	32.1		
Unknown	13.1	12.6	13.2			19.9	20.0	20.0		
Cigarette smoking (cigarettes/day)[b]										
Non-smoker	32.4	32.5	32.4	0.083	6.1	94.1	93.4	94.4	0.333	5.0
Ex-smoker	32.8	31.8	33.3			3.1	3.2	3.0		
Current smoker (<10)	7.1	6.5	7.4			1.9	2.3	1.8		
Current smoker (≥10)	27.7	29.3	27			0.9	1.0	0.8		
Physical activity[b]										
Inactive	45.3	47.1	44.6	0.002	5.3	52.6	54.6	51.8	0.218	6.1
Minimally active	47.3	46.1	47.8			39.2	38.1	39.6		
HEPA	7.4	6.8	7.6			8.2	7.4	8.6		
Adult height (cm)[a]	173.2 (5.6)	173.1 (5.7)	173.3 (5.6)	0.073	2.8	160.4 (5.2)	160.6 (5.2)	160.5 (5.2)	0.562	2.0
Anti-diabetic medications[b]	3.0	2.4	3.2	0.001	5.2	1.2	1.6	1.1	0.191	4.4

Anti-hypertensive medications[b]	8.2	7.1	8.7	<0.001	5.8	3.8	3.6	3.9	0.747	1.1
Anti-dyslipidemic medications[b]	4.5	4.0	4.6	0.037	3.3	2.4	2.6	2.3	0.526	2.2

Data are presented as [a]means (standard deviation), or [b]percentages. P values were for differences between alcohol flushers and non-flushers.
HEPA, health-enhancing physically active; SD, Standardized difference; USD, US dollars.

Table 2. Associations of alcohol flushing status with alcohol consumption and alcohol consumption-related liver enzyme levels among Korean men and women

Alcohol consumption and liver enzyme levels	Men					Women				
	Number of subjects (n=20 696)	Alcohol flushers (n=5940)	Alcohol non-flushers (n=14 756)	P-value	% difference, (95% CI)[c]	Number of subjects (n=3985)	Alcohol flushers (n=1155)	Alcohol non-flushers (n=2830)	P-value	% difference, (95% CI)[c]
Alcohol consumption (g/day)[a]				<0.001					<0.001	
0 g/day	7.6	13.5	5.3			22.8	29.1	20.3		
<8 g/day	39.5	57.6	32.2			66.1	66.6	65.8		
8–30 g/day	37.0	23.8	42.4			9.4	3.7	11.7		
≥30 g/day	15.9	5.1	20.2			1.8	0.6	2.2		
Log transformed alcohol consumption[b]	2.3 (2.2-2.3)	1.6 (1.5-1.6)	2.5 (2.4-2.5)	<0.001		0.7 (0.6-0.7)	0.2 (0.1-0.3)	0.9 (0.8-1.0)	<0.001	
Alcohol consumption (g/day)[c]	9.5 (9.4-9.7)	5.0 (4.8-5.1)	12.1 (11.9-12.3)	<0.001	−59.0 (−60.4 − −57.6)	2.0 (1.9-2.1)	1.2 (1.0-1.3)	2.4 (2.3-2.5)	<0.001	−52.5 (−57.6 − −46.8)
AST (IU/L)[c]	22.3 (22.2-22.4)	21.4 (21.2-21.6)	22.6 (22.5-22.8)	<0.001	−5.5 (−6.5 − −4.5)	17.9 (17.7-18.1)	17.7 (17.8-18.0)	17.9 (17.7-18.1)	0.292	−1.0 (−2.9 − 0.9)
ALT (IU/L)[c]	24.4 (24.3-25.2)	23.2 (22.8-23.4)	25.0 (24.8-25.2)	<0.001	−7.4 (−8.9 − −5.9)	13.6 (13.4-13.8)	13.7 (13.3-14.1)	13.6 (13.3-13.9)	0.626	0.8 (−2.3 − 4.1)
GGT (mg/dL)[c]	33.7 (35.9-36.7)	27.9 (27.5-28.4)	36.3 (35.9-36.7)	<0.001	−22.8 (−24.3 − −21.3)	14.7 (14.5-15.0)	14.1 (13.7-14.5)	15.0 (14.7-15.3)	<0.001	−5.8 (−9.1 − −2.4)
CAC score >0[a]	15.1	14.2	15.5	0.017		3.0	2.3	3.3	0.082	

Data are presented as [a]percentages or [b, c] mean (95% confidence intervals). c Non-normally distributed variables were log transformed and mean differences on the log scale were converted to percentage differences.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CAC, coronary artery calcium; GGT, gamma-glutamyl transferase.

Table 3. Results of the instrumental variable (IV) analysis (using alcohol flushing as an IV) and the multivariable regression analysis, showing the impact of log-transformed alcohol consumption* on cardiovascular risk factors and coronary artery calcium in men.

Variables	IV analysis						Multivariable regression analysis		
	Model 1			Model 2 (adjusted model)					
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value
Body mass index (kg/m^2)	0.194	0.121–0.266	<0.001	0.169	0.097–0.240	<0.001	0.154	0.129–0.180	<0.001
Waist circumference (cm)	0.639	0.447–0.831	<0.001	0.560	0.376–0.744	<0.001	0.388	0.322–0.453	<0.001
Systolic blood pressure (mmHg)	1.398	1.094–1.703	<0.001	1.251	0.951–1.551	<0.001	1.046	0.939–1.153	<0.001
Diastolic blood pressure (mmHg)	1.435	1.185–1.686	<0.001	1.373	1.125–1.620	<0.001	0.937	0.849–1.025	<0.001
HDL-C (mg/dL)	1.516	1.218–1.814	<0.001	1.476	1.182–1.770	<0.001	0.947	0.842–1.052	<0.001
Total cholesterol (mg/dL)	1.885	1.037-2.732	<0.001	2.293	1.459~3.128	<0.001	1.315	1.017–1.613	<0.001
LDL-C (mg/dL)	−0.614	−1.380–0.151	0.116	−0.231	−0.985–0.524	0.5492	−0.163	−0.433–0.107	0.237
Log-transformed triglycerides	0.052	0.040–0.065	<0.001	0.055	0.043–0.068	<0.001	0.030	0.026–0.034	<0.001
Log-transformed fasting glucose	0.015	0.012–0.018	<0.001	0.013	0.010–0.016	<0.001	0.007	0.006–0.008	<0.001
A1C (%)	0.011	−0.001–0.023	0.600	0.003	−0.007–0.013	0.548	−0.001	−0.004–0.003	0.730
Log-transformed insulin	0.021	0.007–0.035	0.004	0.018	0.004–0.032	0.011	−0.004	−0.009–0.001	0.108
Log-transformed HOMA-IR	0.036	0.021–0.052	<0.001	0.031	0.016–0.045	<0.001	0.003	−0.002–0.009-	0.201
CAC score	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
CAC score, binary outcomes									
≥1	1.088	1.015–1.167	0.017	1.109	1.028–1.196	0.008	1.039	1.011–1.067	0.005
CAC score, nominal outcomes: By generalized logistic regression									
0	1.000			1.000			1.000		
1–100	1.077	0.999–1.160	0.053	1.097	1.013–1.187	0.023	1.036	1.008–1.066	0.012
≥101	1.155	0.978–1.365	0.090	1.212	1.013–1.451	0.036	1.060	0.997–1.128	0.062
Overall p-value			0.044			0.015			0.014
CAC score, ordinal outcome: By ordinal logistic regression	1.090	1.017–1.168	0.015	1.114	1.034–1.201	0.005	1.039	1.012–1.066	0.005
Proportional odds asssumption			0.526			<0.001			<0.001

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; A1C, hemoglobin A1c; CAC, coronary artery calcium; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio; IV, instrumental variable.
* IV analysis in model 1was conducted without any adjustment of confounders. In model 2 (adjusted model) of the IV analysis and the multivariable analyses, age, education, monthly household income, cigarette smoking, physical activity, adult height, and medications for diabetes, hypertension, and dyslipidemia were adjusted for. The F-statistics for IV analyses were 2782 in model 1 and 304 in model 2. Log-transformed alcohol consumption was calculated as $\log_2$[(alcohol amount in grams + 1)/8], and was employed because approximately 10% of participants reported no alcohol consumption and the distribution of alcohol consumption among alcohol drinkers was markedly right-skewed. Eight grams of alcohol correspond to one glass of soju, the most popular alcoholic beverage in South Korea.

Table 4. Results of the instrumental variable analysis (using alcohol flushing as an IV) and the multivariable regression analysis, showing the impact of log-transformed alcohol consumption* on cardiovascular risk factors and coronary artery calcium (CAC) in women.

Variables	IV analysis						Multivariable regression analysis		
	Model 1			Model 2 (adjusted model)					
	Beta	95% CI	P-value	Beta	95% CI	P-value	Beta	95% CI	P-value
Body mass index (kg/m^2)	0.111	−0.242–0.463	0.537	0.090	−0.241–0.422	0.594	0.098	0.023–0.173	0.010
Waist circumference (cm)	0.315	−0.601–1.230	0.501	0.327	−0.532–1.186	0.455	0.228	0.033–0.423	0.022
Systolic blood pressure (mmHg)	1.132	−0.209–2.473	0.098	0.953	−0.293–2.198	0.134	0.602	0.320–0.884	<0.001
Diastolic blood pressure (mmHg)	1.460	0.416–2.504	0.006	1.309	0.324–2.294	0.009	0.601	0.379–0.824	<0.001
HDL-C (mg/dL)	1.908	0.287–3.529	0.021	1.865	0.304–3.426	0.019	1.299	0.947–1.651	<0.001
Total cholesterol (mg/dL)	1.169	−2.449–4.789	0.526	0.169	−3.274–3.613	0.923	0.720	−0.060–1.500	0.071
LDL-C (mg/dL)	−0.454	−3.760–2.852	0.788	−1.353	−4.478–1.773	0.396	−0.549	−1.258–0.159	0.128
Log transformed triglycerides	−0.009	−0.060–0.042	0.730	−0.014	−0.061–0.034	0.579	−0.001	−0.012–0.009	0.795
Log transformed fasting glucose	0.009	−0.003–0.021	0.132	0.010	0.001–0.028	0.047	0.002	−0.001–0.004	0.090
A1C (%)	0.001	−0.047–0.045	0.983	0.008	−0.028–0.044	0.683	−0.015	−0.023–0.006	<0.001
Log transformed insulin	−0.057	−0.119–0.005	0.074	−0.045	−0.105–0.015	0.139	−0.013	−0.027–0.001	0.061
Log transformed HOMA-IR	−0.049	−0.117–0.019	0.156	−0.036	−0.101–0.029	0.282	−0.011	−0.026–0.004	0.150
CAC score	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
CAC score, binary outcomes									
≥1	1.849	0.922–3.709	0.083	1.822	0.872–3.806	0.110	1.168	1.005–1.356	0.043

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; A1C, hemoglobin A1c; CAC, coronary artery calcium; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio; IV, instrumental variable, NA, not applicable.

* IV analysis in model 1 was conducted without any adjustment of confounders. In model 2 (adjusted model) of the IV analysis and the multivariable analyses, age, education, monthly household income, cigarette smoking, physical activity, adult height, and medications for diabetes, hypertension, and dyslipidemia were adjusted for. The F-statistics for IV analyses were 189 in model 1 and 31 in model 2. Log-transformed alcohol consumption was calculated as $\log_2$[(alcohol amount in grams + 1)/8], and was employed because approximately 10% of participants reported no alcohol consumption and the distribution of alcohol consumption among alcohol drinkers was markedly right-skewed. Eight grams of alcohol correspond to one glass of soju, the most popular alcoholic beverage in South Korea.